A Research Note

Comparison of Erythorbic and Ascorbic Acids as Inhibitors of Enzymatic Browning in Apple

ABSTRACT

The effectiveness of ascorbic acid (AA) and erythorbic acid (EA) in inhibiting enzymatic browning at cut surfaces of apple and in raw apple juice was determined by tristimulus colorimetry. Red Delicious and Winesap plugs, dipped for 90 sec in 0.8–1.6% solutions of AA or EA, showed longer lags before the onset of browning with the former compound. AA and EA were similar in effectiveness in apple juice. Because the relative effectiveness of AA and EA depends on the system in which they are compared, they should not be used interchangeably as sulfite alternatives without experimental verification of equivalence.

INTRODUCTION

RECENT CONCERN over the danger to some asthmatics posed by the use of sulfites to control enzymatic browning in cut fruits and vegetables has created a demand for sulfite substitutes (Taylor and Bush, 1986; Andres, (1985). Most of the sulfite alternatives that have been marketed are formulations of citric acid with L-ascorbic acid (AA), a well established browning inhibitor (Bauernfeind and Pinkert, 1970), or its isomer erythorbic acid (EA). AA has been reported to be a more effective inhibitor of enzymatic browning than EA (Borenstein, 1965; Bauernfeind and Pinkert, 1970). Nevertheless, recommended use concentrations of the two reducing agents are similar (Anonymous, 1977). The objective in the present study was to determine whether equivalent concentrations of EA and AA were equally effective in controlling enzymatic browning in apple.

MATERIALS & METHODS

WINESAP AND RED DELICIOUS apples were obtained from local food stores in the spring of 1986 and 1987, stored briefly at 4°C until needed, and then equilibrated for 1 hr at room temperature prior to use. All procedures for sample preparation, colorimetry and data analysis are described in detail in an earlier publication (Sapers and Douglas, 1987). Briefly, individual apples were cut in half along the stem axis, and four plugs were bored from each half with a 22 mm diameter stainless steel cutting tube. Each plug was cut transversely at its midpoint, yielding two half-plugs sharing a common cut surface, one to be dipped in a solution of AA or EA in water (pH 2.6-2.9) or 1% citric acid (pH 2.1) for 90 sec, and the other, a control, to be dipped in water or 1% citric acid for 10 sec to remove adhering juice. Treatment solutions contained 0.8 or 1.6% (w/v) AA or EA, equivalent to 45.4 or 90.8 mM, concentrations previously found to be partially effective in inhibiting browning (Sapers and Douglas, 1987). These, rather than higher concentrations, were used so that differences in the degree of inhibition among treatments could be observed. Two concentrations of AA and EA could be compared in duplicate with the eight plugs obtained from a single apple. Colorimetry was performed with a Gardner XL-23 tristimulus colorimeter with a 19 mm aperture, standardized against a white tile. Plugs were placed over the open aperture so as not to disturb the treated surface. Between reflectance measurements, plugs were held at room temperature in covered crystallizing dishes to minimize dehydration. L- and a-values (decreasing L and increasing a being associated with browning) were recorded at frequent intervals over 6 hr and again after 24 hr and plotted against log time, yielding linear or bilinear curves with an initial region of zero slope. The lag time (time before the onset of browning) corresponding to this region was located at its intersection with the linear region of the curve. Lag times could be estimated to within ± 1-2 min for brief lags (< 1 hr) and to within ± 5–10 min for long lags (2–7 hr) because of the logarithmic time scale. The slope of the linear portion was determined by linear regression. The extent of browning in treated and control plugs was determined by the change in L (△ L) or a (△ a) over a specified time interval. The overall effectiveness of a browning inhibitor was determined from the difference between control and treatment △ values, expressed as a percentage of the control △ values, an index we call the percent inhibition. The magnitude of this index indicated the degree of browning inhibition (positive values) or promotion (negative values). The significance of differences in percent inhibition, lag time, and slope values between corresponding EA and AA treatments was determined for each trial by subdividing the sums of squares for treatments into specific contrasts (Cochran and Cox, 1957). This approach was followed because of the dependence of the treatment response on the tendency of the fruit to brown, which varied from trial to trial.

AA and EA also were compared in the raw juice from Golden Delicious or Granny Smith apples (duplicate trials), prepared with an Acme Supreme Juicerator (Model 6001). At zero time (within 1 min of juice preparation), 25 mL aliquots of juice were mixed in cylindrical optical cells (57.1 mm i.d.) with 1 mL of the following solutions: H_2O (control), 0.406% (w/v) $NaHSO_3$ (equivalent to 96 ppm SO_2 in the juice) and 0.125 or 0.250% (w/v) AA or EA (equivalent to 0.27 or 0.54 mM in the juice, respectively). L- and a-values were measured with the tristimulus colorimeter at frequent intervals over 90 min; initial lag times and percent inhibition values at different storage times were determined from these data. Because of rapid changes in all samples except the SO_2 treatment, inhibition calculations for each treatment were based on the difference between L- and a-values at a given time and the 1.5 min values for the SO_2-treated juice, which approximated a zero-time measurement. Lag times for juice could be estimated to within ± 2 min over the entire time period. The significance of differences in percent inhibition values between corresponding EA and AA treatments of juice was determined by ANOVA.

RESULTS & DISCUSSION

COMPARISONS of percent inhibition values for Winesap and Red Delicious plugs, examined at 2, 6, and 24 hr following treatment with AA or EA, in water or in 1% citric acid, indicated that AA was consistently more effective than EA (Table 1). The negative inhibition values obtained with some EA treatments reflect the tendency of low concentrations of EA (and to a lesser extent, AA) to enhance the discoloration of samples that are undergoing severe browning. The statistical analysis of these data was complicated by extensive variability in the response of individual apples to treatment which was probably indicative of variation in polyphenol oxidase activity and/or phenolic composition. However, even where significance could not be demonstrated

Table 1—Inhibition of enzymatic browning at cut surface of apple plugs with erythorbic acid (EA) and ascorbic acid (AA)

Cultivar	Dip composition	Conc (mM)	Trial	% Inhibition[z] 2 hr EA	2 hr AA	6 hr EA	6 hr AA	24 hr EA	24 hr AA	Lag (min)[y] EA	Lag (min)[y] AA	Slope[x] EA	Slope[x] AA
Winesap	EA or AA in H_2O	45.4	1	17*[w]	56	12	26	−30	−2	20	26	2.3	1.5
			2	−28*	56	−24*	40	−36*	28	16	25	3.4*	1.7
		90.8	1	12*	100	4*	82	−24*	42	32	90	4.2*	0.8
			2	55	90	45	76	15	48	30	62	1.8	1.0[v]
	EA or AA in 1% citric acid	45.4	1	34*	82	16*	66	−10	14	23	40	2.2*	0.7[v]
			2	24*	88	10*	72	−22	9	20	115	2.8	1.4
		90.8	1	95	101	78	94	18	56	42*	240	0.3	0.5
			2	92	95	80	84	40	20	145	210	0.8	1.2[v]
Red Delicious	EA or AA in H_2O	45.4	1	−28	5	−27	−4	−4	8	25	42	3.0	3.0
			2	0	30	−5	15	11	−10	34	50	2.6	1.9
		90.8	1	8	86	−4	32	12	18	43	86	3.2	1.6
			2	−30*	116	−51*	82	−24*	33	30*	180	2.5	1.3
	EA or AA in 1% citric acid	45.4	1	29*	96	0*	44	−11	0	20	105	2.6	1.8
			2	62*	100	30*	84	6	43	30	200	1.1	1.7
		90.8	1	94	104	66	89	16	36	88*	265	0.9[v]	1.4[v]
			2	98	101	69	80	26	42	145	180	1.3	0.8

[z] (Δ a control − Δ a treatment) × 100 ÷ Δ a control; Δ values are differences in a-value between 1 min and specified times.
[y] Times before onset of browning, obtained from a-value vs log time curve.
[x] Slope of linear portion of a-value vs log time curve; correlation coefficient for regression ≥ 0.96 except where otherwise noted.
[w] Asterisk indicates that means for a given EA-AA comparison are significantly different at $p < 0.05$ by ANOVA.
[v] Correlation coefficient between 0.90 and 0.95.

Table 2—Inhibition of enzymatic browning in apple juice with erythorbic acid (EA) and ascorbic acid (AA)

Cultivar	Concn (mM)	Lag (min)[z,x] L EA	Lag (min)[z,x] L AA	Lag (min)[z,x] a EA	Lag (min)[z,x] a AA	Storage (min)	% Inhibition[y,x] L EA	% Inhibition[y,x] L AA	% Inhibition[y,x] a EA	% Inhibition[y,x] a AA
Golden Delicious	0.27	0	0	0	0	30	20	22	28	28
						60	10	12	14	15
	0.54	22	22	22	22	30	54	54	71	70
						60	33	32	40	38
Granny Smith	0.27	10	10	10	10	30	35	44	46	52
						60	14	18	25	25
	0.54	40	45	45	45	30	70	69	78	75
						60	50	48	62	58

[z] Time corresponding to intersection of initial zero slope region and linear change in L- or a-value vs time curve.
[y] (Δ control − Δ treatment) × 100 ÷ Δ control; Δ values are differences between L- or a-values at 30 or 60 min for each treatment and L- or a-value for SO_2 treatment at 1.5 min.
[x] Mean of duplicate trials.

statistically, trends clearly could be seen. With Winesap, the higher percent inhibition values obtained for AA treatments were a consequence of longer lag times as well as lower browning rates, as indicated by the browning curve slopes. With Red Delicious, the difference between AA and EA was due primarily to the longer lag times obtained with the former compound. Inhibition data based on measurements of L (not shown) were similar to those based on a-values given in Table 1.

In contrast to the cut surface data, comparisons of lag time values for Golden Delicious and Granny Smith juice containing 0.27 or 0.54 mM EA or AA, indicated that the two compounds were equivalent as browning inhibitors (Table 2). Similarly, differences in percent inhibition between corresponding EA and AA treatments, based on changes in L- and a-values over 30 and 60 min, were not significant, as tested by ANOVA. Inagaki et al (1963) reported that EA and AA had almost equal potency for preventing color change in apple juice.

The striking difference between the apple cut surface and juice systems may be related to the rate of oxidation of EA and AA, which would affect the duration of the lag period (Ponting and Joslyn, 1948). EA has been reported to undergo copper-catalyzed oxidation more rapidly than AA both in aqueous model systems and food products (Borenstein, 1965). The higher surface-to-volume ratio of the thin film of juice at cut apple surfaces, compared to that of the bulk juice, would favor oxidation in the former system. Enzyme-catalyzed oxidation of EA and AA may be more important in the bulk juice than at cut surfaces because of the extensive disruption of apple tissue and release of enzymes during juice preparation. Cucumber ascorbic acid oxidase has been reported to oxidize EA as rapidly as AA (McCarthy et al., 1939).

Disagreements in the literature over the relative effectiveness of EA and AA as browning inhibitors may have resulted from differences in the choice of system used to evaluate these compounds or in the concentration ranges compared. Because the performance of EA and AA as browning inhibitors is highly dependent on the system being protected, we suggest that one compound not be substituted for the other in the formulation of sulfite substitutes without prior experimentation to verify their equivalence.

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Ms received 6/5/87; revised 7/16/87; accepted 7/16/87.